Received: 6 May 2020 | Revised: 2 September 2020 | Accepted: 3 October 2020
DOI: 10.1002/jbm.b.34737

ORIGINAL RESEARCH REPORT

Quantitative assessment of dentine mineralization and tubule occlusion by NovaMin and stannous fluoride using serial block face scanning electron microscopy

Behrad Mahmoodi[1] | Patricia Goggin[2] | Christabel Fowler[3] | Richard B. Cook[1]

[1]National Centre for Advanced Tribology at Southampton (nCATS), Faculty of Engineering and Physical Science, University of Southampton, Southampton, UK

[2]Biomedical Imaging Unit, University Hospital Southampton, Southampton, UK

[3]GlaxoSmithKline Oral Health Research and Development, Weybridge, UK

Correspondence
Behrad Mahmoodi, Faculty of Engineering and Physical Science University of Southampton, Room 3011, Building 5, Highfield, Southampton SO17 1BJ, UK.
Email: b.mahmoodi@soton.ac.uk

Abstract

Dentine hypersensitivity (DH) is one of the most common dental conditions affecting most adults during their lifetime. Tubule occlusion is a widely accepted method for treating DH. Current in-vitro techniques such as focused ion beam, scanning electron microscopy (SEM), or hydraulic conductance that are used to determine tubule occlusion do not provide the depth of occlusion, are time-consuming, expensive and the volume of dentine tested is limited. The presented study aimed to assess the ability of serial block-face SEM (SBF-SEM) to section dentine, to quantify the number of occluded tubules including the depth of penetration by NovaMin and stannous fluoride (SnF_2) and to compare mineral density between the control and treated dentine. Results demonstrated that NovaMin provided a better occlusion with 100% of the tubules blocked at the surface compared to 83% for SnF_2. The grayscale value (230.42) was significantly higher ($p \leq 0.05$) after treatment with NovaMin compared to SnF_2 (222.06) and the control (196.37), indicating increased mineral density and dentine mineralization. SBF-SEM has the potential to be used for large volume analysis of bone-like materials at high resolution with minimal sample preparation over a short period. It can be significantly useful in the development and research of new biomaterials.

KEYWORDS

mineralization, NovaMin®, serial block-face SEM, stannous fluoride, tubule occlusion

1 | INTRODUCTION

Teeth can become sensitive for several reasons such as cracks, dental decay, and deep fillings. However, two of the main causes of dentine hypersensitivity (DH) are gum recession and thinning of the enamel due to over brushing or acid erosion, exposing dentine tubules. Tubules run through the entire structure of the dentine to the pulp and are filled with dentinal fluid. Stimuli such as pressure, temperature, and osmosis can alter the fluid flow within the tubules, distorting the nerve in the pulp. This initiates a neurological response resulting in a sharp pain experienced by individuals.[1] In the early stages, it is possible to manage and reduce sensitivity symptoms by using dentifrices designed to occlude these tubules by either depositing a layer over the exposed surface or by plugging materials down the tubules.[2] Stannous fluoride (SnF_2)[3–5] and NovaMin[6–8] are two of the active ingredients used in toothpastes to occlude tubules and treat sensitivity symptoms. NovaMin releases calcium and phosphate ions when it comes in contact with saliva, these ions get deposited onto the dentine surface and form a layer that is chemically and structurally similar to hydroxyapatite.[9] SnF_2 rapidly hydrolyses in the presence of saliva, fluoride ions are released and get incorporated into the hydroxyapatite structure forming a fluorapatite.[10] The stannous ions are then oxidized from Sn (II) to Sn (IV) and form insoluble oxides which plug the tubules.[5] He et al[4] demonstrated that SnF_2 dentifrice provided an immediate and significant sensitivity relief in a clinical study compared to the sodium monofluorophosphate control. An in-vitro study by Takamizawa et al[5] showed significant tubule occlusion after treatment with SnF_2. Earl et al[11] used a focused ion beam (FIB) and scanning electron microscopy (SEM) to investigate the depth of tubules occlusion after treatment with NovaMin. The results showed that dentine tubules were occluded to approximately 1 μm (the depth of FIB cut). It is extremely time-consuming to mill a large volume of material to high depths by FIB-SEM. Earl et al[12] used FIB-SEM to study the structure of the dentine where only 15 tubules were managed over 20 hr. SEM, on the other hand, does not provide information about the depth of occlusion. Serial block-face SEM (SBF-SEM) is a 3D imaging technique that uses a diamond knife in a microtome sitting within the chamber of an SEM to cut nanometer-sized slices from the surface of a sample (block face). An image is initially taken from the block face, the sample is then cut at a given depth by the microtome, exposing a new block face. This process continues automatically and resulting images are compiled to provide a 3D reconstruction of the sample.[13] The present study aims to determine the level of tubule occlusion by NovaMin and SnF_2, both by determining the number of occluded tubules per unit volume but also the depth of penetration of the active ingredients using SBF-SEM. It also aims to use the backscattered images from the SBF-SEM to investigate the mineralization potential of the two ingredients using the grayscale values of the images which are proportional to the mineral density.[14]

2 | MATERIALS AND METHODS

2.1 | Dentin discs and toothpaste treatment

Bovine dentine discs (n = 6) with a thickness of 1.00 ± 0.3 mm were supplied by Modus Laboratories Ltd (Reading, UK). Each disc was polished using 3 and 1 μm diamond suspensions to get a smooth surface finish, etched with 1% citric acid for 30 s to remove the smear layer and washed with deionized water for 1 min. The discs were then randomly divided into two equal treatment groups (n = 3). Discs were halved with one half treated with either Sensodyne Repair and Protect (NovaMin) or Rapid Relief (SnF_2) toothpastes (Table 1) and one half as control; 2.0 ± 0.1 mg of respective toothpaste was weighed onto a medium manual toothbrush (Colgate) and brushed on to the dentine discs for 2 min, twice per day for 7 days. About 0.50 ml of artificial saliva (AS) with a pH of 6.5 was pipetted on to the discs to stimulate the reaction of active ingredients in conditions that were more representative of the oral environment. The control discs were only brushed with AS. All specimens were kept in AS between brushings. The AS contained carboxymethylcellulose (5 g), glycerol (50 g), DI (1 L) sodium phosphate dibasic dodecahydrate (0.58 g), urea (1 g), NaCl (0.4 g), KCl (0.4 g), and CaCl2 (0.6 g).[15]

2.2 | SBF-SEM imaging and processing

After the 7 days of brushing treatment, dentine discs were air-dried at room temperature (21–23°C, 24 hr), and 0.5 by 0.5 mm blocks were cut out from the discs. The blocks were infiltrated with resin and polymerized at 60°C overnight (Agar low viscosity [ALV] resin, Agar Scientific, Stansted, UK). The excess resin was trimmed away and the block glued on to aluminum pin using conductive glue before sputter coating with gold/palladium to increase the conductivity of the block. Blocks were imaged using a Gatan 3View (Gatan, Abingdon, UK) inside a FEI Quanta 250 FEGSEM (Thermo Fisher, Eindhoven, the Netherlands) at 2.5 kV accelerating voltage, and with a vacuum level of 30 Pa. Stacks of 6,000 × 6,000 pixel images were collected at a pixel size of 19 nm resulting in a 114 × 114 μm field of view. Six hundred slices were cut by an ultrasonic diamond knife with a thickness of 60 nm during data acquisition resulting in an imaging depth of approximately 36 μm. After each slice, the dentine surface was imaged with a backscatter (BS) electron detector. Once the images were collected they were processed and analyzed using ImageJ software. All tubules (open or occluded) were identified using the segmentation function, their position was subsequently recorded throughout the stack and it was used to align the data set and straighten each tubule. Once the tubules were identified and straightened, the level of the blockage was evaluated by scanning through the core of each one and recording the grayscale values (Figure 1). As demonstrated in the

TABLE 1 Toothpastes and their active ingredient used in this study

Toothpaste	Active ingredient	Other ingredients	Manufacturer
Sensodyne Repair and Protec	NovaMin (calcium sodium phosphosilicate) 5% wt/wt	Glycerin, PEG-8, hydrated silica, cocamidopropyl betaine, sodium methyl cocoyl taurate, aroma, titanium dioxide, carbomer, sodium saccharin, sodium fluoride (1,450 ppm)	GlaxoSmithKline
Sensodyne Rapid Relief	Stannous fluoride (SnF_2), 0.454% wt/wt	Glycerin, PEG-8, hydrated silica, pentasodium triphosphate, aroma, sodium lauryl sulfate, titanium dioxide, carbomer, stannous fluoride, cocamidopropyl betaine, sodium saccharin, sodium fluoride, limonene., sodium fluoride 0.072% wt/wt (1,450 ppm fluoride).	GlaxoSmithKline

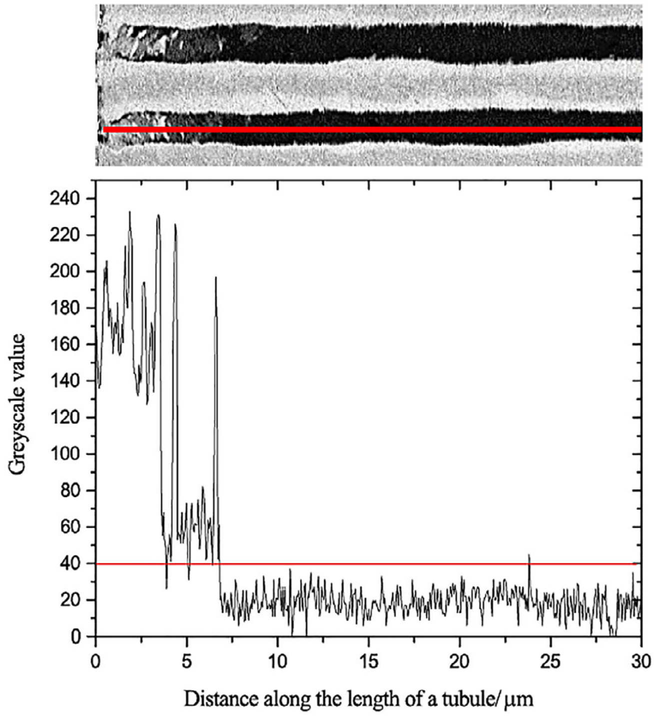

FIGURE 1 XZ and YZ planes for a tubule and gray values taken by scanning the core of tubule

example given in Figure 1 there is a big drop in grayscale value to below 40 at around 7 μm when the line moves past the occluded portion of the tubule. Therefore, this value was chosen as the benchmark to separate filled versus unfilled tubules.

2.3 | Remineralization potential and diameter of tubule opening

To compare the mineral density of the dentine samples the grayscale values of the backscattered electron (BSE) images were taken from the block face by ImageJ software. The grayscale values from 100 random areas around the tubules were recorded at every 3 μm from the surface to the depth of 30 μm. After each tubule was identified, a stack of all images for that tubule was made and a thickness graphic mask was applied to generate a heat map. The diameter of these heat objects was then measured throughout the stack which represented the open section of the tubule. Areas that had a grayscale value of more than 40 (occluded) were thresholded to appear black and excluded from the measurement. The outputted values included the highest, lowest, mean and *SD* diameter throughout the entire stack.

2.4 | Statistics

The data was initially analyzed using a one-way analysis of variance (ANOVA) to determine significance. This was followed by a *t* test (assuming equal variance) to identify any significant differences between the means of the groups; ($p \leq 0.05$) was considered to be significant.

3 | RESULTS

3.1 | Tubule occlusion and mineralization

The SEM images (Figure 2) taken before slicing showed that both toothpastes were successful in occluding tubules compared to the control samples. NovaMin formed a layer over the tubules (Figure 2b) whereas there was no layer formed by SnF_2 (Figure 2c). Cross-sections of the tubules (Figure 2, right hand images) showed that the tubules within the control group were empty. Tubules treated with NovaMin and SnF_2 contain occluding material throughout the length of the tubules at various depths. The level of tubule occlusion reduced as the distance from the surface increased and at 3 μm, the percentage of tubules with occlusion was significantly ($p \leq 0.05$) reduced for both treatment groups. Although NovaMin had better occlusion at the surface SnF_2, occluded more tubules between 3 and 24 μm from the surface. However, between 24 and 30 μm NovaMin had a superior occlusion rate although the difference was nonsignificant ($p \geq 0.05$) (Figure 3L). Grayscale values around the tubules (Figure 3R) were significantly higher ($p \leq 0.05$) for NovaMin-treated samples at the surface and 30 μm below the surface (230.42 and 213.55, respectively) compared to SnF_2 (222.06 and 192.76) and the control (196.37, 192.35).

3.2 | Tubule diameter

The diameter of tubule opening (Figure 4L) was significantly ($p \leq 0.05$) reduced from 0.79 ± 0.05 to 0.66 ± 0.21 μm after treatment with NovaMin. No significant ($p \geq 0.05$) change was seen after SnF_2 treatment. Figure 4R was taken from a NovaMin-treated sample after it had been through a cutting and imaging process (30 μm was removed from the surface). The growth of material around the tubule is evident (red arrow) and can explain the reduction in the diameter of tubule opening.

4 | DISCUSSION

For prevention and treatment of DH the permeability of the tissue needs to be reduced to inhibit stimuli from altering the fluid flow and stimulating the nerve endings inside of the tooth. This can be achieved by occlusion of the exposed dentine tubules. In the present in-vitro study, SBF-SEM was used to compare dentine tubule occlusion by Sensodyne Repair and Protect and Rapid Relief toothpaste containing NovaMin and SnF_2, respectively as active ingredients to treat DH. The BSE imaging also made it possible to compare the mineral density of the dentine tissues between the control and the treated samples. SBF-SEM imaged approximately 100 tubules per sample with a 19 × 19 × 60 nm voxels size over approximately 12 hr.

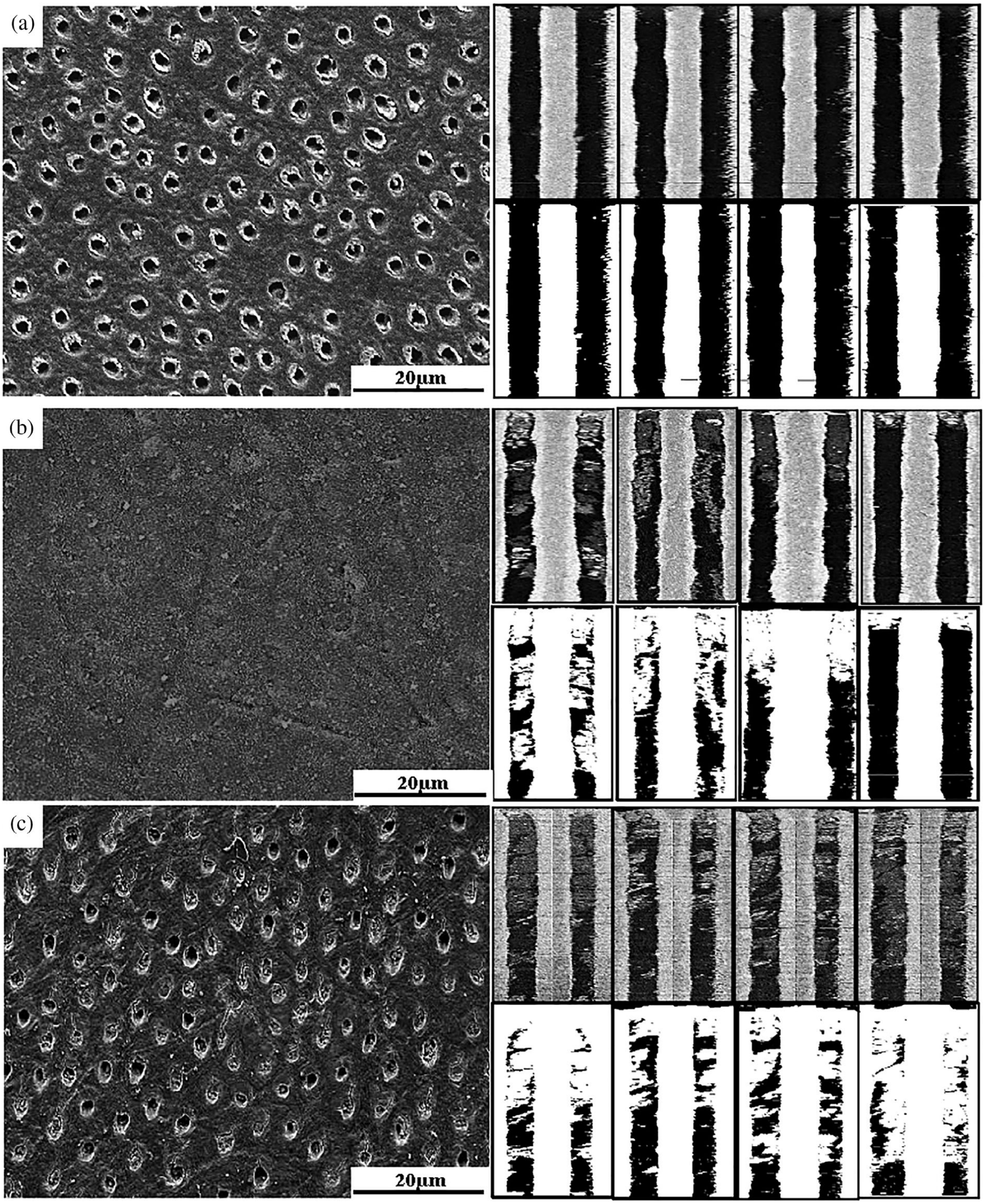

FIGURE 2 Scanning electron microscopy (SEM) images of control (a), NovaMin (b), and SnF_2 (c) treated dentine surface, images to the right are the cross section of random tubules taken from the respective groups. Top row: each pair shows XZ (left) and YZ (right) planes for a tubule. Bottom row: tubule was 3D-flood-filled from the bottom

This resulted in an imaging volume of approximately 4.4355 × 105μm^3. The SEM data showed that both materials were capable of occluding a significant number of tubules, while in the control group they were still open. However, the occlusion percentage decreased with increasing depth away from the surface. NovaMin produced a better level of occlusion both at the surface and at 30 μm. It also resulted in a reduction in tubule opening by forming a layer down the walls of the tubules. The grayscale values around the tubules were higher for NovaMin-treated samples at each given depth compared to the control and SnF_2. These results are similar to studies in literature,

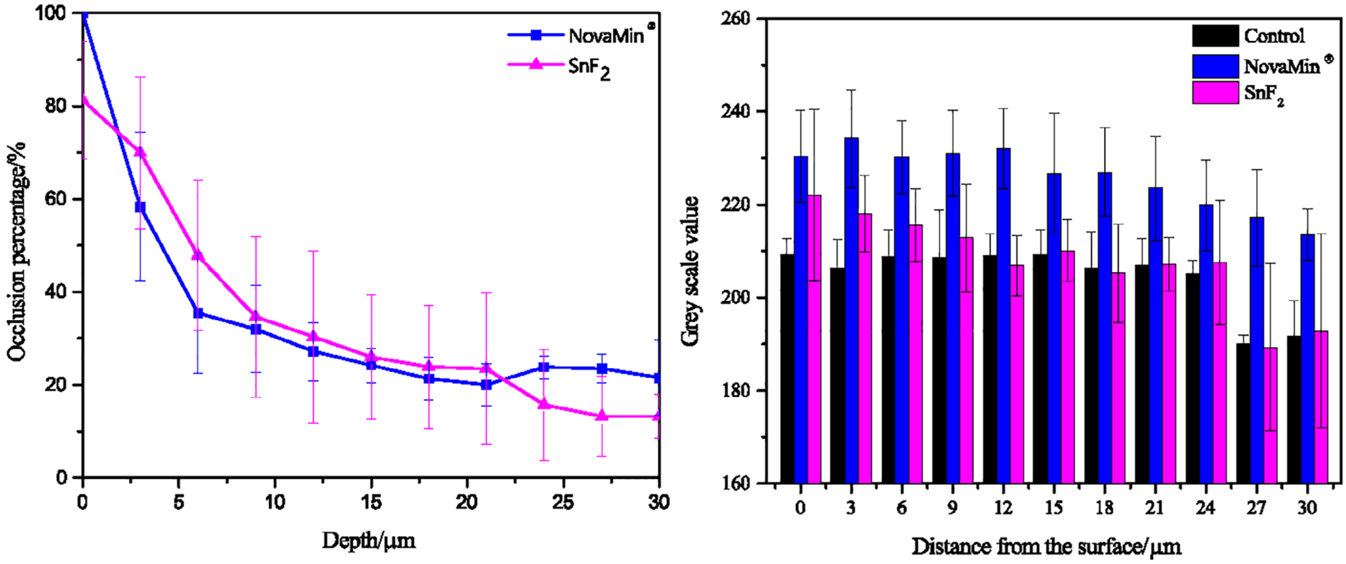

FIGURE 3 Percentage of blocked tubules (L), grayscale values of control and treated dentine (R)

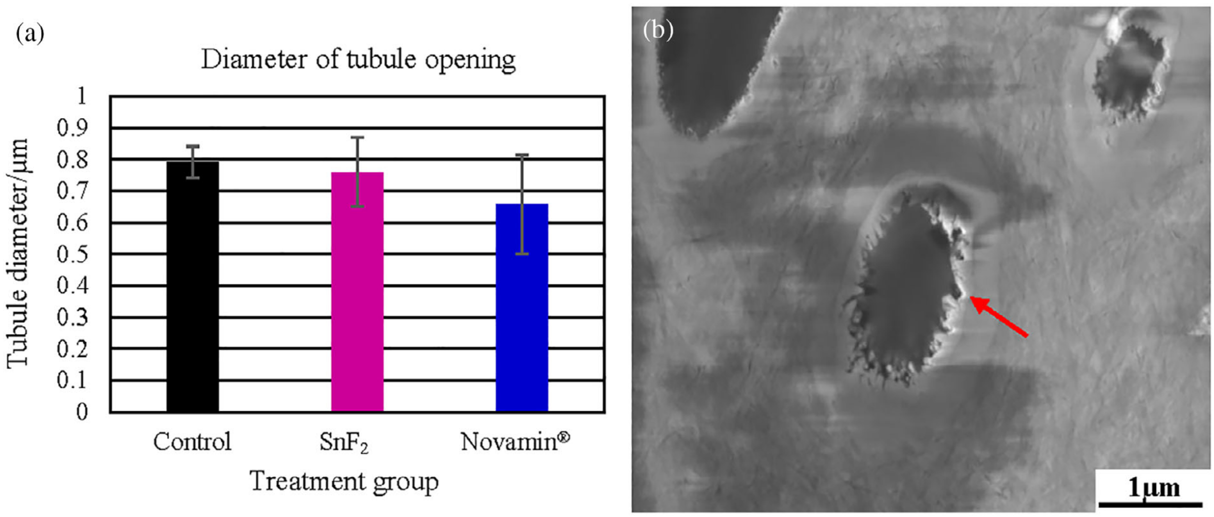

FIGURE 4 Diameter of the tubule opening (L) and scanning electron microscopy (SEM) image taken from a NovaMin-treated dentine disc after 30 μm was removed from the surface (R)

Parkinson [16] used SEM to investigate the occlusion and mineralization of NovaMin and SnF_2. They reported that NovaMin containing toothpaste had a significantly higher level of dentin tubule occlusion and mineralization to compare to SnF_2 after being brushed for 2 min twice per day over 4 days. An in-vitro SEM study by Kulal et al[17] reported 98.1% dentine tubule occlusion following a 7 day (2 min per day) treatment with a NovaMin containing toothpaste. Whereas another in-vitro SEM study reported 92.73% occlusion after brushing with NovaMin containing toothpaste for 6 min twice a day for 7 days.[18] A recent in-vitro SEM study by Hines et al[19] reported that SnF_2 containing toothpaste occluded 82% of tubules. A clinical study comparing NovaMin and SnF_2 for treating DH also reported that NovaMin reduced DH significantly more than SnF_2 after 12 weeks.[20] The findings in this study encourage the use of NovaMin containing toothpaste over a toothpaste with SnF_2 as an active ingredient due to a better occlusion and mineralization potential of NovaMin. Also, SBF-SEM enabled the study of a much larger volume of dentine over a short period with high resolution. To analyze a similar number of tubules at this resolution with other techniques such as FIB SEM would have required significantly more sample preparation which would be time-consuming, costly and the depth would be limited to the FIB slice.

5 | CONCLUSION

NovaMin and SnF_2 containing dentifrice have the ability to occlude dentine tubules, with NovaMin able to occlude 100% of the tubules due to penetration of material into the tubules and the formation of the surface layer. The NovaMin and SnF_2 both increased the dentine mineralization level as quantified by grayscale values from the backscattered images. The increased level of tubule occlusion and dentine mineralization by the NovaMin containing dentifrice may provide better relief and protection from dental hypersensitivity.

SBF-SEM is a high resolution imaging technique which can provide a new quantitative method of assessing dentine tubule occlusion depth and percentage of occluded tubules, while also allowing for the assessment of mineralization changes within the treated tissues.

ACKNOWLEDGMENTS

The authors kindly acknowledge; GlaxoSmithKline, Weybridge, UK for funding the research, the authors would like to thank all the staff at the University of Southampton's Biomedical Imaging Unit for access to the 3View SEM and their help with the imaging, Dr David Chatelet (Biomedical Imaging unit, University of Southampton) for assistance with image processing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

B.M. contributed to conception, design, data acquisition and interpretation, drafted, and critically revised the manuscript. P.G. contributed to design, data acquisition, and critically revised the manuscript. C.F. contributed to conception, design, and critically revised the manuscript. R.B.C. contributed to conception, design, analysis and interpretation, and critically revised the manuscript. All authors gave their final approval and agree to be accountable for all aspects of the work.

ORCID

Behrad Mahmoodi https://orcid.org/0000-0002-1558-1149

REFERENCES

1. Porto, I. C. C. M., Andrade, A. K. M., & Montes, M. A. J. R. (2009). Diagnosis and treatment of dentinal hypersensitivity. *J Oral Sci*, *51*, 323–332. https://doi.org/10.2334/josnusd.51.323
2. Arnold, W. H., Prange, M., & Naumova, E. A. (2015). Effectiveness of various toothpastes on dentine tubule occlusion. *J Dent*, *43*, 440–449. https://doi.org/10.1016/j.jdent.2015.01.014
3. Blong, M. A., Volding, B., Thrash, W. J., & Jones, D. L. (1985). Effects of a gel containing 0.4 percent stannous fluoride on dentinal hypersensitivity. *Dent Hyg (Chic)*, *59*, 489–492.
4. He, T., Barker, M. L., Qaqish, J., & Sharma, N. (2011). Fast onset sensitivity relief of a 0.454% stannous fluoride dentifrice. *J Clin Dent*, *22*, 46–50.
5. Takamizawa, T., Tsujimoto, A., Ishii, R., Ujiie, M., Kawazu, M., Hidari, T., … Miyazaki, M. (2019). Laboratory evaluation of dentin tubule occlusion after use of dentifrices containing stannous fluoride. *J Oral Sci*, *61*, 276–283. https://doi.org/10.2334/josnusd.18-0176
6. Mahale, S., Badade, P., & Panjwani, A. (2015). Dentinal tubule occlusion by desensitizing dentifrices: SEM study. *J Dent Med Sci*, *14*, 21–24. https://doi.org/10.9790/0853-141012124
7. Reynolds, E. C. (2008). Calcium phosphate-based remineralization systems: scientific evidence? *Aust Dent J*, *53*(3), 268–273. https://doi.org/10.1111/j.1834-7819.2008.00061.x
8. Venkatesh Babu, N. S., Dave, P. N., Bhanushali, P. V., Moureen, Y., Shah, S., & Kiran, Y. C. (2017). Comparative analysis of remineralizing potential of three commercially available agents- an in vitro study. *IOSR J Dent Med Sci*, *16*, 1–5. https://doi.org/10.9790/0853-1602030105
9. Gupta, P., Jan, S. M., & Behal, R. (2019). In vitro evaluation of dentinal tubule occlusion by novamin (bioactive glass). *Int J Dev Res*, *09*, 26934–26938.
10. Miller, S., Truong, T., Heu, R., Stranick, M., Bouchard, D., & Gaffar, A. (1994). Recent advances in stannous fluoride technology: antibacterial efficacy and mechanism of action towards hypersensitivity. *Int Dent J*, *44*, 83–98.
11. Earl, J. S., Leary, R. K., Muller, K. H., Langford, R. M., & Greenspan, D. C. (2011). Physical and chemical characterization of dentin surface following treatment with NovaMin technology. *J Clin Dent*, *22*, 62–67.
12. Earl, J. S., Leary, R. K., Perrin, J. S., Brydson, R., Harrington, J. P., Markowitz, K., & Milne, S. J. (2010). Characterization of dentine structure in three dimensions using FIB-SEM. *J Microsc*, *240*, 1–5. https://doi.org/10.1111/j.1365-2818.2010.03396.x
13. Smith, D., & Starborg, T. (2019). Serial block face scanning electron microscopy in cell biology: applications and technology. *Tissue Cell*, *57*, 111–122. https://doi.org/10.1016/j.tice.2018.08.011
14. Bloebaum, R. D., Skedros, J. G., Vajda, E. G., Bachus, K. N., & Constantz, B. R. (1997). Determining mineral content variations in bone using backscattered electron imaging. *Bone*, *20*, 485–490. https://doi.org/10.1016/S8756-3282(97)00015-X
15. Nakamichi, I., Iwaku, M., & Fusayama, T. (1983). Bovine teeth as possible substitutes in the adhesion test. *J Dent Res*, *62*, 1076–1081. https://doi.org/10.1177/00220345830620101501
16. Parkinson, C. R., & Willson, R. J. (2011). A comparative in vitro study investigating the occlusion and mineralization properties of commercial toothpastes in a four-day dentin disc model. *J Clin Dent*, *22*, 74–81.
17. Kulal, R., Jayanti, I., Sambashivaiah, S., & Bilchodmath, S. (2016). An in-vitro comparison of nano hydroxyapatite, novamin and proargin desensitizing toothpastes - a SEM study. *J Clin Diagn Res*, *10*, 51–54. https://doi.org/10.7860/JCDR/2016/18991.8649
18. Reddy, G. V., Akula, S., Malgikar, S., Babu, P. R., Reddy, G. J., & Josephin, J. J. (2017). Comparative scanning electron microscope analysis of diode laser and desensitizing toothpastes for evaluation of efficacy of dentinal tubular occlusion. *J Indian Soc Periodontol*, *21*, 102–106. https://doi.org/10.4103/jisp.jisp_153_17
19. Hines, D., Xu, S., Stranick, M., Lavender, S., Pilch, S., Zhang, Y. P., … Williams, M. (2019). Effect of a stannous fluoride toothpaste on dentinal hypersensitivity: in vitro and clinical evaluation. *J Am Dent Assoc*, *150*, 47–59. https://doi.org/10.1016/j.adaj.2019.01.006
20. Sharma, N., Roy, S., Kakar, A., Greenspan, D. C., & Scott, R. (2010). A clinical study comparing oral formulations containing 7.5% calcium sodium phosphosilicate (novamin®), 5% potassium nitrate, and 0.4% stannous fluoride for the management of dentin hypersensitivity. *J Clin Dent*, *21*(3), 88–92.

How to cite this article: Mahmoodi B, Goggin P, Fowler C, Cook RB. Quantitative assessment of dentine mineralization and tubule occlusion by NovaMin and stannous fluoride using serial block face scanning electron microscopy. *J Biomed Mater Res*. 2020;1–6. https://doi.org/10.1002/jbm.b.34737